REMARKS

Reconsideration is requested.

Claims 1-106 have been canceled, without prejudice. Claims 107-135 are pending.

The Examiner's detailed action is noted with appreciation and the applicants have amended the claims above in an effort to reduce the issues for appeal, without prejudice. Specifically, with regard to the claim objections noted in paragraphs 9-12 on pages 3-4 of the Office Action dated May 5, 2005, the claims have been amended to "clarify the record" with regard to the mutations recited in the adenylate kinase of the claims. See, page 3, last sentence, of the Office Action dated May 5, 2005. Moreover, the Markush groups of claims 108, 120 and 123 have been amended as suggested by the Examiner in paragraph 10 on page 4 of the Office Action dated May 5, 2005. Claim 109 has been amended in a manner which is believed to overcome the objection stated in paragraph 11 on page 4 of the Office Action dated May 5, 2005. Moreover, the Examiner's helpful suggestion in paragraph 12 on page 4 of the Office Action with regard to claim 128 has been incorporated in the above amendments. Withdrawal of the claim objections noted on pages 3-4 of the Office Action dated May 5, 2005, is requested.

The Section 112, second paragraph, rejection of claims 107-112 and 115 is believed to be obviated by the above amendments. To the extent the rejection is not obviated by the above amendments, the rejection is traversed and reconsideration and withdrawal of the rejection are requested in view of the following comments.

Specifically, with regard to the Examiner's comments in paragraph 13(a), the claims have been revised to specifically indicate that the method produces a luciferase which is substantially free of enzymatically active adenylate kinase however the applicants believe such an amendment should not be required as the applicants believe the term "adenylate kinase" requires an enzymatically active enzyme. The claims have been amended however to reduce the issues for appeal, without prejudice.

With regard to the Examiner's comments in paragraph 13(b), the applicants believe the claims are definite and the Examiner has demonstrated as much by reference to specific documents which are available to one of ordinary skill in the art. As noted on page 9 of the applicants' Amendment of February 14, 2005, the Examiner is believed to have acknowledged in point (5) on page 4 of the Office Action of May 2, 2003, that certain embodiments were enablement described by the specification. Specifically, the 354 mutation of *Photinus pyralis* in the equivalent Luciola mutation, are believed to have been recognized as having been supported on the basis at least as a reference in the specification of WO 95/25798. The specification also includes a reference to European Patent Application No. 92110808.0, which is published as EP-A-524448, which is a patent application corresponding to the cited Kajiyama reference, such that the applicants believe that these proteins are known in the art and the meets and bounds of the claimed invention will be understood.

The dependency of claim 112 has been amended above in response to the Examiner's comments in paragraph 13(c) of the Office Action dated May 5, 2005.

Moreover, the reference to temperature in claim 112, 122 and 130 has been amended in response to the Examiner's comments in paragraph 13(d) of the Office Action.

Similarly, claim 117 has been revised in response to the Examiner's concerns in paragraph 13(e) of the Office Action dated May 5, 2005.

Claim 117 has been revised in response to the Examiner's comments in paragraph 13(f), of the Office Action.

Claims 117, 126 and 133 have been revised in response to the Examiner's comments in paragraph 13(g). The claims are submitted to be definite.

The claims have been revised in response to the Examiner's comments in paragraph 13(h). The Examiner's helpful suggestions are acknowledged with appreciation.

Finally, the claims have been revised in response to the Examiner's comments in paragraph 13(i).

The claims are submitted to be definite and withdrawal of the Section 112, second paragraph, rejection of claims 107-112 and 115, is requested.

The Section 112, first paragraph, rejection of claims 108-109, 115-116 and 120-135 stated in paragraph 14 of the Office Action dated May 5, 2005, is traversed. Reconsideration and withdrawal of the rejection are requested as the Examiner's assessment of, for example, the disclosure of WO 95/25798, appears to be contrary to the Patent Office's examination of the related U.S. application which issued as U.S. Patent No. 6,132,983, with the following claims:

1. An isolated protein having luciferase activity which protein has over 60% amino acid sequence homology to the luciferase from Photinus pyralis, Luciola mingrelica, Luciola cruciata, or Luciola lateralis and includes the amino acid sequence XGDDKPGA wherein X is an amino acid residue other than glutamate, glycine, proline or aspartic acid.

2. An isolated protein as claimed in claim 1 which includes the amino acid sequence TPXGDDKPGA where X is an amino acid residue other than glutamate, glycine, proline or aspartic acid.

3. An isolated protein as claimed in claim 1 wherein X is selected from the group consisting of tryptophan, valine, leucine, isoleucine and asparagine or an analogue or modification of any of these.

4. An isolated protein as claimed in claim 1 wherein X is selected from the group consisting of lysine and arginine or an analogue or modification of any of these.

5. An isolated protein as claimed in claim 1 wherein said protein is a firefly or glow-worm protein.

6. An isolated DNA encoding a protein as claimed in claim 1.

7. A vector comprising a luc gene encoding for a protein as claimed in claim 1.

8. An isolated protein according to claim 1 wherein said protein is a Photinus or Luciola luciferase.

9. An isolated DNA according to claim 6 having the sequence of SEQ ID NO: 1.

10. An isolated DNA encoding a protein as claimed in claim 2.

11. An isolated DNA according to claim 6 wherein said protein is a luciferase from one of Photinus or Luciola.

12. A vector of claim 7 selected from the group consisting of pKK223-3, pDR540 and pT7-7 into which said luc gene has been ligated.

13. A vector comprising a DNA according to claim 6.

14. A vector comprising a DNA according to claim 9.

15. A vector comprising a DNA according to claim 10.

16. A vector comprising a DNA according to claim 11.

17. An isolated cell comprising a vector according to claim 7.

18. An isolated cell capable of expressing a protein according to claim 1.

19. A cell according to claim 17 wherein said cell is an E. coli, S. cerevisiae or insect cell.

20. A cell according to claim 18 wherein said cell is an E. coli, S. cerevisiae or insect cell.

21. An assay method comprising measuring ATP using luciferin and a protein of claim 1 to generate light, the quantity of which is related to the amount of ATP present.

22. An assay according to claim 21 wherein said assay is carried out at a temperature of from 30.degree. C. to 70.degree. C.

23. An assay according to claim 22 wherein said temperature is in the range of 37.degree. C. to 60.degree. C.

24. An assay according to claim 23 wherein said temperature is in the range of 40.degree. C. to 50.degree. C.

25. A test kit comprising a specific binding reagent labeled with a protein according to claim 1.

26. A method of increasing the heat stability of a protein having luciferase activity, which protein has over 60% amino acid sequence homology to the luciferase from Photinus pyralis, Luciola mingrelica, Luciola cruciata, or Luciola lateralis, and comprises an amino acid sequence XGDDKPGA, said method comprising replacing X with an amino acid, analogue or modification thereof which is different from glutamate, glycine, proline, or aspartic acid.

27. In a luciferase which has over 60% amino acid sequence homology to the luciferase from Photinus pyralis, Luciola mingrelica, Luciola cruciata, or Luciola lateralis, is thermally stable to a temperature of about 30.degree. C. and comprises an amino acid sequence XGDDKPGA, the improvement comprising an amino acid other than glutamate, glycine, proline or aspartic acid as a replacement for X.

28. An isolated and purified protein comprising the amino acid sequence of SEQ ID NO:2 wherein Xaa is one of tryptophan, valine, leucine, isoleucine, asparagine, lysine and arginine or an analogue or modification of these.

29. An isolated and purified DNA encoding a protein of claim 28.

30. A vector comprising a DNA of claim 29.

31. An isolated and purified cell comprising the vector of claim 30.

32. An assay method comprising measuring ATP using luciferin and luciferase to generate light, the quantity of which is related to the amount of ATP characterized, said luciferase being a protein according to claim 28.

Specifically, the Patent Office has examined the disclosure WO95/25798 and previously found that the specification is enabling for, and describes, at least the broad range of proteins having luciferase activities which are recited in the claims of the patent. Reconsideration and withdrawal of the Section 112, first paragraph, rejection of claims 108-109, 115-116 and 120-135 stated in paragraph 14 of the Office Action dated May 5, 2005, are requested.

The Section 112, first paragraph rejection of claims 107-135 stated in paragraph 15 of the Office Action dated May 5, 2005, is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following comments.

The Examiner appears to believe that the specification fails to describe a representative number of species of claimed genus of proteins. The Examiner's comments on page 11 of the Office Action dated May 5, 2005, are noted in this regard. The Examiner is requested to again however see the attached copy of U.S. Patent No. 6,132,983, which issued by the U.S. Patent Office from the disclosure of WO 95/25798. The Examiner's apparent limited assessment of the disclosure of WO 95/25798 as described on page 11 of the Office Action of May 5, 2005, appears therefore to be inconsistent with the Patent Office's issuance of U.S. Patent No. 6,132,983. The Examiner will appreciate that the claims of U.S. Patent No. 6,132,983 are presumed to be valid.

Withdrawal of the Section 112, first paragraph, rejection of claims 107-135 stated in paragraph 15 of the Office Action dated May 5, 2005, is requested.

The Section 112, first paragraph, rejection of claims 107-135 stated in paragraph 16 of the Office Action dated May 5, 2005, is traversed. Reconsideration and withdrawal of the rejection are requested.

For completeness, the applicants note that the wild type sequences of the recited proteins are well-known in the art, such as is evidenced by the attached Ye et al, BBA 1339 (1997) 39-52. Moreover, it will be appreciated by one of ordinary skill that the thermostability of proteins can be enhanced by mutation and the references mentioned in the specification submitted previously provide examples of the same. A person of ordinary skill in the art would therefore well understand and appreciate the proteins as cited in the claimed invention.

The Examiner is requested to appreciate that the presently claimed invention provides a procedure whereby mutation is used to modify properties which are ultimately used as a basis for purification to remove particularly troublesome contaminants. The claimed invention is submitted to be adequately described in the present specification. Withdrawal of the Section 112, first paragraph, rejection of claim 107-135, is requested.

The applicants submit that the presently claimed invention requires a combination of elements which are well-known and/or identified or obtainable by mere routine experimentation at the time the presently claimed invention was made. That is, the applicants have discovered a means to recombinantly produce luciferase, or other desired products, in the absence of the essential enzyme adenylate kinase, or other essential cellular components. In a larger sense however the applicants have discovered that desired proteins may be recombinantly produced by the claimed method and using the claimed cells, in the substantial absence of proteins which are otherwise undesired but have an activity that is essential for survival of the host cell or for a viable production process using the host cell.

The present specification describes examples of thermostable polypeptide products (i.e., luciferase proteins). Moreover, the art, such as Kajiyama (Biochemistry 32:13795-13799, of record) teaches a vector encoding a mutant thermostable luciferase wherein the mutation resulting in the thermostable luciferase is a substitution of Threonine with isoleucine at position 217. The applicants further note WO 95/25798 (Lowe et al.), further teaches that the thermal and pH stability and the specific activity of the enzyme described by Kajiyama were increased. See, the paragraph spanning

pages 1 and 2 of Lowe et al. Lowe et al. further teach that luciferases having increased heat stability over wild-type luciferases or produced from luciferases of each of *Photinus pyralis, Luciola mingrelica, Luciola lateralis and Luciola cruciata*. See, page 2 of Lowe et al. Lowe et al further teach alternative forms of the mutated luciferases may include changes to amino acid sequence corresponding to amino acid 217 of the Luciola firefly luciferase or 215 of Photinus pyralis changed to a hydrophobic amino acid, such as isoleucine, leucine or valine, as described in EP 052448. See, page 3, second full paragraph of Lowe et al. The Examiner is also requested to see the above claims of the corresponding U.S. Patent No. 6,132,983.

Lowe et al further describes luciferases wherein the mutations at positions 217 or 215 are included in combination with the mutation at position 354. Lowe et al describes the methods for isolating and identifying further luciferases which are thermostable and/or pH insensitive and for a method of using the same, such as in recombinant methods. Lowe et al further provides sequence information from which one of ordinary skill in the art may make and use aspects of the presently claimed invention without further experimentation.

The description of Lowe et al is an example of the advanced level of the ordinary skill in the art at the time the presently claimed invention was made. The applicants should not be required to describe in the present application that which was known to one of ordinary skill in the art for the teaching, for example, Lowe et al.

Further luciferase mutants are described in WO 96/22376 (Squirrell et al., of record). Specifically, the noted amino acids 217, 215, 354 and 356 of Lowe et al are described in Squirrell et al. Further mutations containing double and triple amino acid

changes are also described. See, page 5, first paragraph of Squirrell et al. As with Lowe et al, Squirrell et al is a representative of the level of ordinary skill in the art at the time the presently claimed invention was made and the applicants should not be required to describe in the present application that which is well known in the art.

Belinga et al (of record) describes standard technique for isolating and purifying luciferases and the applicants respectfully submit that identification of use of a variety of specific species having the desired characteristics of the present claims would have been routine to one of ordinary skill in the art once given the present specification. Beyond the identification and natural sources, the applicants note that Kajiyama (U.S. Patent No. 5,229,285) teaches that mutant genuses of genes encoding wild-type firefly luciferases can be effected according to methods known in the art. See, column 2, lines 52-54 of Kajiyama. Further details of recombinant technology are also describes throughout Kajiyama.

With regard to the description of proteins which hinder the use of the desired polypeptide product and have activities essential for survival the host cell or a viable production process using the host cell, such as adenylate kinase, the Examiner is believed to have admitted that Gilles et al (PNAS, Vol. 83, pp 5798-5802, August 1986) teaches thermosensitive mutants of E. Coli with the mutation in the endogenous *adk* gene encoding adenylate kinase. See, page 9 of Paper No. 30. The applicants submit that further such polypeptide could be made, without undue experimentation, by recombinant means described, for example, in Kajiyama (U.S. Patent No. 5,229,285), and with the general knowledge of one of ordinary skill in the art at the time of the present invention.

Moreover, Liang et al (Gene 80 (1989) 21-28) teaches the efficient cloning of a mutant adenylate-kinase-encoding gene from *E. Coli*. Liang et al teaches that cloning of the wild-type *adk* gene was reported as early as 1995 and that multiple mutants had apparently been obtained which contains thermolabile adenylate kinase as early as 1986. See, page 22, left column of Liang et al.

The applicants respectfully submit therefore that methods and material which may be required to produce the protein which hinder the use of a polypeptide product recited in the present claims which also has an activity essential for survival of a host cell or for a viable production process used in a host cell, such as adenylate kinase, as presently claimed, were well-described in the art and available to one of ordinary skill in the art at the time the present invention was made. A more detailed description in the present application of further species falling within the claims should not be required to satisfy the requirement of Section 112, first paragraph.

Withdrawal of the Section 112, first paragraph, rejection of claims 107-135 stated in paragraph 16 of the Office Action dated May 5, 2005, is requested.

The applicants acknowledge with appreciation, the Examiner's recognition that the claims are patentable over the previously-cited combination of EP 373962 (Backman), Delinga (Journal of Chromatography A, 695:33-40 (1995)), Gilles (PNAS, 83:5798-5802, 1986) and Kajiyama (Biochemistry 32:13795-13799, 1993). See, paragraph 17 on page 20 of the Office Action dated May 5, 2005.

The Section 103 rejection of claims 107-108, 110-115 and 120-124 over Backman, Squirrell (WO 96/02665), Squirrell (WO 96/22376) and Gilles, as stated in paragraph 18 of the Office Action dated May 5, 2005, is traversed. Reconsideration and

withdrawal of the rejection are requested in view of the following distinguishing comments.

As previously-described in the record, the presently claimed invention requires a combination of the use of the desired protein which is stable under given conditions and undesired protein which is unstable under the same conditions wherein the undesired protein is one which hinders the use of the desired protein that has activities essential for the survival of the host cell or for a viable production process using a host cell. Exemplified desired proteins include luciferase and undesired proteins are exemplified in the present application as by adenylate kinase. The Examiner has combined the noted references through an inappropriate use of hindsight.

The results of Backman relate to the use of thermostable enzymes wherein a thermostable form of an enzyme is used in a recombinant production method followed by application of extreme heat to denature all but the desired peptide. See, column 2, line 53 through column 3, line 17 of Backman. Backman does not teach luciferase production.

More importantly, the process of Backman does not require production or engineering of proteins or polypeptides as required by the presently claimed invention. That is, a luciferase produced according to method of Backman would only require production of thermostable forms of luciferase in a mesophilic host cell, culturing the mesophilic host cell to produce the thermostable luciferase and purify the thermostable luciferase by at least heating to a temperature sufficient to inactivate the unwanted contaminants but not sufficient to inactivate the thermostable luciferase. See, column 2, lines 21-37 of Backman.

Backman does not describe or suggest the simultaneous production of a desired polypeptide in a mutant form which has increased tolerance to a particular reaction condition, such as pH or temperature, as well as a mutant form or an undesired protein which has a decreased tolerance to the reaction condition as compared to a wild-type form of the undesired protein and wherein the undesired protein hinders the use of the polypeptide product and has an activity that is essential for survival of a host cell or for a viable production process using a host cell. Backman therefore provides a process wherein the identification or use of a mutant of the undesired peptide of the presently claimed invention would not be required. The combination of Gilles and/or either of the Squirrell references cited by the Examiner, with Backman therefore would not be logical to one of ordinary skill in the art wishing to produce luciferase in the process of Backman.

Reconsideration and withdrawal of the Section 103 rejection of claims 107-108, 110-115 and 120-124 stated in paragraph 18 of the Office Action dated May 5, 2005, are requested.

The Section 103 rejection of claims 117-119 and 125-127 stated in paragraph 19 of the Office Action dated May 5, 2005, is traversed. Reconsideration and withdrawal of the rejection are requested as the Examiner's secondary references (i.e., Novagen and Kiel) fails to cure the deficiencies noted above with regard to Backman and Squirrell and Gilles.

The applicants further submit that the cited references would not motivate one of ordinary skill to have made the presently claimed invention. To a certain extent, the applicants believe the cited references would not have been considered by one of

ordinary skill in the art to be analogous art. Specifically, Backman is believed to relate to the field of enzymes obtainable from thermophilic organisms, such as those required for use in techniques such as PCR. Squirrell however is concerned with different types of enzymes, with different functions from different sources, such that the enzymes of Squirrell possess different properties. Luciferases are thermosensitive enzymes obtainable from sources such as fireflies and used in signaling systems. Gilles is believed to relate to yet another field and is a scientific paper relating to a structure-activity relationship of yet another different enzyme (i.e., adenylate kinase). The Novagen and Kiel references discuss techniques used in biotechnology generally however there are not believed to be anything in the cited art which would have motivated one of ordinary skill in the art to combine the references in the manner asserted by the Examiner to have made the presently claimed invention.

Reconsideration and withdrawal of the Section 103 rejection of claims 117-119 and 125-127 stated in paragraph 19 of the Office Action dated May 5, 2005, are requested.

The Section 103 rejection of claims 107, 109-114, 116 and 128-132 over Backman in view of Squirrell (1), Kajiyama (Biochemistry 32:13795-13799) and Gilles stated in paragraph 20 of the Office Action dated May 5, 2005, is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above as well as the following distinguishing comments.

Beyond the above noted deficiencies of Backman, the applicants further note that Backman does not describe or suggestion a simultaneous production of a desired polypeptide in a mutant form which has increased tolerance to a particular reaction

condition, such as pH or temperature, as well as a mutant form of an undesired protein which has decreased tolerance to the reaction condition as compared to a wild-type form of the undesired protein and wherein the undesired protein hinders the use of the polypeptide production and has an activity that is essential for survival of a host cell or for a viable production process using the host cell. Backman therefore provides a process wherein the identification or use of a mutant of the undesired polypeptide of the presently claimed invention will not be required. The combination of Gilles, which describes the identification of adenylate kinase, with Backman therefore would not be logical to one of ordinary skill in the art wishing to produce luciferase in the process of Backman.

Kajiyama teaches a mutant thermostable luciferase and also that "one of the most important goals of protein engineering is to produce mutant enzymes which have greater thermostability than the parent protein". See, page 13795, left column, second full paragraph of Kajiyama. One of ordinary skill in the art reading Kajiyama and Backman would, at best, be motivated to produce luciferases which could be sufficiently thermostable to be expressed in a mesophilic host cell of Backman. The preferred temperatures of purification according to Backman is 80°C to 95°C. See, column 3, lines 14 and 15 of Backman. The thermostable mutant luciferase of Kajiyama was tested at 50°C and found to have a half-life which was roughly 8-10 times longer than that of the wild-type luciferase. See, page 13796, right column on the first full paragraph, and Figure 3 of Kajiyama. Even if one of ordinary skill in the art reading Kajiyama and Backman would have been motivated, at least, to produce further luciferase mutants with even greater thermostability, such is not the subject of the

presently claimed invention. The further teaching of Squirrell (1), is not believed to cure the deficiencies of the combination of Backman, Kajiyama and Gilles.

Withdrawal of the Section 103 rejection of claims 107, 109-114, 116 and 128-132 stated in paragraph 20 of the Office Action of May 5, 2005, is requested.

The Section 103 rejection of claims 117-119 and 133-135 over Backman, Squirrell (1), Kajiyama, Gilles, Novagen and Kiel, stated in paragraph 21 of the Office Action dated May 5, 2005, is traversed. Reconsideration and withdrawal of the rejection are requested as the further cited references of Novagen and Kiel fail to cure the above-noted deficiencies of the other recited references.

Withdrawal of the Section 103 rejection of claims 117-119 and 133-135 stated in paragraph 21 of the Office Action dated May 5, 2005, is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned if anything further is required in this regard.

Consideration of the references listed on the attached PTO-1449 Form and return of an initialed copy of the same pursuant to MPEP § 609, are requested.

Respectfully submitted,

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